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EFFECTS OF PHARMACOLOGIC INTERVENTION ON OXYGENATION, LUNG WATER AND PROTEIN LEAK IN THE PSEUDOMONAS ARDS PORCINE MODEL

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We have shown that a combination of antihistamine and cyclooxygenase blockade is effective in attenuating the lung injury manifested as increased capillary permeability and hypoxemia in the porcine ARDS model. We have also shown that a Platelet Activating Factor receptor blocker is effective attenuating the early phase of the injury. Present and future studies involve characterization of various cytokines such as Tumor Necrosis Factor, the interleukins and long and short lived oxidants which have all been implicated in the syndrome. Eventual goal of these studies will be the prevention or reversal of the evolving syndrome by combination pharmacological blockade targeted at specific inflammatory mediators.

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SUMMARY

The adult respiratory distress syndrome is a condition which occurs as a result of both direct and indirect pulmonary injury. The mortality rate for the syndrome which may affect previously fit patients is over 50% and higher where sepsis predominates. This mortality, despite modern techniques in intensive care, has hardly changed in 20 years. The pathophysiological changes in the condition result in respiratory failure requiring endotracheal intubation and mechanical ventilation, the appearance of diffuse fluffy infiltrates on chest roentgenogram, a normal pulmonary wedge pressure, an arterial:alveolar pO_2 ratio $\leq$ 0.2, and a total static lung compliance of $\leq$ 50 ml H_2O.

At the cellular level, the lung injury is due to damage of the alveolar capillary membrane by various circulating elements of the blood, most notably neutrophils. These neutrophils become "activated" when they come in contact with a soluble or phagocytic stimulus, break down and release many inflammatory mediators. Among these mediators are the products of the cyclooxygenase and lipoxygenase systems of arachadonic acid breakdown such as the prostaglandins, leukotrienes, SRS-A, the complement factors C5a, C3a, oxygen-free radicals, and tumor necrosis factor.

All of these substances are toxic to the alveolar capillary membrane and eventually cause its disintegration with concomitant protein leak across the damaged membrane into the lung. When the lymphatic clearance capacity of the lung is exceeded, pulmonary edema occurs and the clinical picture seen in ARDS unfolds. Pseudomonas-induced ARDS in the porcine model has been used as a representative example of the syndrome in this laboratory.

Because ARDS is mediated by numerous inflammatory mediators, it is likely that treatment will require several pharmacological blocking agents. We have already established that treatment with cimetidine, or ranitidine in combination

with diphenhydramine, H_2 and H_1 blockers, respectively, and ibuprofen, a prostaglandin antagonist, given i.v. at 20 and 120 minutes after pseudomonas infusion, significantly attenuates both the early hypertensive and late permeability phases of the syndrome as measured by hemodynamic parameters, blood gases and slope index (the ratio of change of radioactivity between the heart and right lung) and measurement of the extravascular lung water by the indicator dilution technique.

An anti-platelet activating factor, SRI 63-675, has been shown to attenuate the early phase of pulmonary hypertension and possibly improve the late permeability changes in the model but caused severe hemolysis, thus making it impractical for clinical use at this time. Experiments using superoxide radical scavengers in the model have not been shown to be effective in improving the response to injury in any way. Results, however, are inconclusive and will need further study.

Measurements of conjugated dienes in serum as a reflection of oxygen radical activity on lipid membranes in the pseudomonas injured animals do not at this time appear to be sufficiently accurate and consistent to use as a marker of the effects of radical scavengers.

Kinetic and quantitative studies of superoxide production from neutrophils separated from the pre- and post-injury phases in the model indicate that pseudomonas primes these cells to produce O_2^- at a much higher rate post-injury than pre-injury, thus implicating neutrophil generated superoxide anion in the endothelial cell damage which occurs. Alveolar macrophages retrieved from bronchoalveolar lavage in the post-injured phase appear also to be capable of generating large quantitites of superoxide anion and hydrogen peroxide and may have a role in the injury process.

Tumor necrosis factor generated by macrophages appears to be increased in the injured animal. This substance has been implicated in many inflammatory processes and has been seen by others to increase in humans exposed to endotoxin.

Static and dynamic compliance in the pseudomonas-treated animals decreases significantly following onset of the bacterial infusion and remains decreased throughout the study period. This parameter will be an important tool in the assessment of further therapeutic manipulations in the model.

FOREWORD

In conducting the research described in this report, the investigators adhere to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. NIH 78-23, Rev. 1978).

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PROBLEM

The adult respiratory distress syndrome (ARDS), as first described by Ashbaugh (1) 20 years ago, is a pathophysiological pulmonary condition of multiple etiologies. The syndrome may be initiated by direct pulmonary injury or may be seen as the lungs' response to a remote or systemic insult. In civilian life, the most common causes of ARDS are multiple trauma, aspiration of gastric contents, sepsis and pancreatitis. In combat soldiers, the condition known as the traumatic wet lung syndrome during the Korean conflict and Da-Nang lung in the Viet Nam war, and now recognized as ARDS, results from blast injuries, direct lung contusion, burn inhalation, inhalation of toxic substances, aspiration, multiple transfusions and as a complication of sepsis. As such, the condition often affects previously fit and healthy patients with a considerable mortality.

Clinically, patients are considered to have the syndrome when certain criteria are met: respiratory failure requiring endotracheal intubation and mechanical ventilation, the appearance of diffuse pulmonary infiltrates on chest roentgenogram, an initial pulmonary wedge pressure of 18 mmHg or less, an arterial to alveolar PO_2 ratio $\leq$ 0.2 and a total static lung compliance of $\leq$ 50 ml H_2O which increases the work of respiration with stiffer lungs and decreases oxygenation.

Applying these criteria, between 150,000 and 200,000 patients in the United States are affected annually by this syndrome. The mortality rate remains at 50% or higher where sepsis predominates (2). The mainstay of treatment is supportive therapy, treatment of the underlying disorder, the maintenance of adequate oxygenation with mechanical ventilation and positive end-expiratory pressure (PEEP), fluid balance, nutrition and antibiotics where indicated, since patients with ARDS are more susceptible to nosocomial infections.

BACKGROUND

Despite the multiple causes of ARDS, the final pathways end in the same result, i.e., damage to the pulmonary capillary membrane with increased permeability and accumulation of water and protein rich fluid in the pulmonary interstitium. Alveolar flooding occurs when interstitial and lymphatic clearance capacity are exceeded, leading to decreased pulmonary compliance and the perfusion of unventilated alveoli manifested as hypoxemia which is refractory to increased inspired oxygen concentrations.

It is likely that successful treatment of ARDS will involve intervention to prevent capillary endothelial damage and protein leak across the membrane, as well as methods to increase compliance and improve oxygenation.

The use of radioisotopically tagged tracer proteins has provided an important technique for the estimation of lung leak in recent years. In this laboratory the use of radiolabeled ^{99m}technetium human serum albumin as a tracer protein across the damaged alveolar capillary membrane has been used in dogs given intravenous oleic acid (3) and pigs given intravenous live Pseudomonas. The method has been used in clinical trials with success to differentiate between cardiogenic and noncardiogenic pulmonary edema (4).

This method of the determination of pulmonary leak along with the measurement of extravascular lung water by the indicator dilution technique has been used in all the animal experiments in this laboratory where such determinations were necessary for the assessment of therapeutic intervention in experimental ARDS. In addition to these methods of determining lung leak, we have added the technique of bronchoalveolar lavage to further study cell traffic across the damaged alveolar capillary membrane and to recover inflammatory and resident cells from the alveolus in order to study their role in the development of the syndrome.

RATIONALE

Role of Inflammatory Mediators in ARDS

It is clear that the lung injury in ARDS is mediated by a large number of substances. That some of these substances are inter-related and share a common final pathway or common enzyme systems is becoming more obvious. What is not clear are the exact inter-relations between these mediators. It is likely that therapeutic intervention with a specific or a combination of specific agents will attenuate the lungs' response to injury and thereby minimize its consequences.

Central to the lungs' response to injury in endotoxin induced ARDS are the neutrophils (5-7). Sequestration of neutrophils takes place soon after endotoxin infusion *in vivo*. The exact method of neutrophil aggregation is not known but it is hypothesized that substances such as complement C3a and C5a, leukotrienes and various other chemotactic substances are involved in the initiation of the process (8). *In vitro*, plasma activated with zymosan causes neutrophil aggregation. Neutrophils become "activated" when they come in contact with a soluble or phagocytic stimulus and manifest this activation as a respiratory burst with an increase in oxygen consumption, activation of the hexose monophosphate shunt and generation of reactive oxygen species and their metabolic products. These products are injurious to cell membranes as well as deactivating enzymes and causing mutagenesis by their action on DNA.

As well as oxygen-free radicals, the neutrophils, platelets, monocytes and lymphocytes can release a number of other factors which have an affect on pulmonary hemodynamics and directly on the endothelium. The products of arachadonic acid metabolism produced by the circulating elements in the blood are thought to cause the acute pulmonary hypertension seen immediately after endotoxin infusion as well as increased lung lymph flow (9-11). Products of the cyclooxygenase system of arachadonic acid metabolism are thought to cause

these effects since increased plasma levels of TxB_2 and 6 Keto $PGF_{1\alpha}$ are temporally related to the initial pulmonary hypertension and increased lung lymph flow. It has been shown by previous experiments in this laboratory that these effects can be blunted by a combination of an anti-prostaglandin in conjunction with histamine receptor blockers (12). The macrophage has been implicated in the lung injury by its release of substances such as the interleukins and tumor necrosis factor, or cachectin (13,14).

The second phase of the lungs' response to endotoxin is characterized by a sustained but lower elevation in pulmonary artery pressure and an increased protein rich lymph flow secondary to an increased capillary permeability. Gamma scintigraphic analysis of radiolabeled tracer across the alveolar capillary membrane, developed in this laboratory, has been helpful in assessing the degree of pulmonary leak in experimental and clinical ARDS as well as the response to therapeutic intervention.

This late phase of pulmonary hypertension and protein leak is not thought by some to be due to the metabolites from the arachadonic cascade; however, it is possible that oxygen-free radicals generated by neutrophils (15) are responsible for a large part of the vascular endothelial cell damage as well as proteolytic enzymes released by degranulating neutrophils (16) all of which result in increased permeability.

Oxygen-free radicals produced by neutrophils are normally converted to non-injurous substances by the enzymes superoxide dismutase and catalase. However, when these mechanisms are overcome by overwhelming oxygen radical production, intermediates such as O_2^-, H_2O_2 and OH ions occur. These are injurious to cells. The hydroxyl radical particularly causes peroxidation of lipid membranes and in the presence of transition metals such as copper and iron these membranes disintegrate, lose their integrity and result in increased permeability (17).

Another seemingly potent mediator in the lung injury seen with endotoxin induced ARDS is the inappropriately named platelet activating factor (PAF). This phospholipid, which is secreted by many mammalian cells and tissues, has been identified as 1-0-alkyl-2-acetyl-sn-glycero-3 phosphorylcholine. PAF aggregates and stimulates both leukocytes and platelets and contracts pulmonary artery and airway smooth muscle in many species (18,19).

EXPERIMENTAL METHODS

1. The Model

The porcine model was used in all the ensuing experiments. Young swine weighing between 14-30 kgs were anesthetized with intramuscular ketamine hydrochloride 25 mg/kg and placed supine. The animals were then given intravenous sodium pentobarbital (10 mg/kg). Following intubation with a cuffed endotracheal tube, they were paralyzed with continuous intravenous pancuronium bromide (0.2 mg/min) to permit mechanical ventilation with 0.5 FiO_2, 5 cm H_2O positive end expiratory (PEEP) and 20 cc/kg tidal volume at a rate which produced a $PaCO_2$ of approximately 40 torr at the beginning of the experiment.

Catheters were inserted into the left common carotid artery for monitoring systemic arterial blood pressure (SAP) and arterial blood gases, and into the right and left external jugular veins for infusion of Pseudomonas (Ps), ^{99m}technetium-labeled serum albumin (TcHSA) and the therapeutic agents to be studied. A thermodilution Swan-Ganz catheter was passed through the right jugular vein into the pulmonary capillary and wedged in position with the balloon inflated. This was used to monitor pulmonary artery pressure (PAP), pulmonary wedge pressure (PWP), and thermodilution cardiac output. Cardiac output was converted to cardiac index (CI) by the formula:

$$CI = \frac{CO}{0.112\ BW\ 2/3}$$

where BW is the body weight in kg. Blood gases were measured with a blood gas analyzer (Instrumentation Laboratories, Model 113).

A 5 French femoral artery lung water catheter (American Edwards Laboratories, Model 96-020-5F) was passed into the lower abdominal aorta for measurement of thermal cardiogreen extravascular lung water (EVLW). In this technique 10 ml of iced, green dye solution (2 mg indocyanine green dye in 10 ml 5% dextrose) were injected as a bolus through the proximal port of the Swan-Ganz catheter as blood was simultaneously withdrawn through the thermistor-tipped femoral artery catheter and a densitometer cuvette (Waters Instruments Inc., Model 402A) linked to a lung water computer (American Edwards Laboratories, Model 9310). The computer measured the mean transit times of the intravascular dye (MTD) and freely diffusable thermal component (MIT) as well as the cardiac output (CO). EVLW was calculated by the formula:

$$EVLW = \frac{CO\ (MTD-MIT)}{BW\ (kg)}$$

Computerized gamma scintigraphy as described above was used as an indicator of pulmonary capillary permeability through the measurement of pulmonary transcapillary albumin flux. Lung-heart radioactivity ratios were constructed with a VAX 8600 computer, and the slope index was calculated by least squares linear regression analysis.

For the purposes of the following studies, a less lethal model than that used previously was employed by infusion of live pseudomonas aeruginosa, PAO strain, 5×10^8 CFU/ml at 0.3 ml/20kg/min, for 1 hour rather than by continuous infusion throughout the entire study period. In Ps control animals this has been shown to produce a marked physiological deterioration, representative of acute ARDS, resulting in an immediate significant increase in PAP which persists

throughout the entire duration of the experiment. SAP shows a progressive decline as does CI and PaO_2. EVLW and SI become significantly elevated when compared to saline controls.

We have already established in a previous study that treatment with a combination of cimetidine 150 mg, diphenhydramine 10 mg/kg and ibuprofen 12.5 mg/kg (CID) given intravenously at 20 and 120 minutes after continuous pseudomonas infusion significantly improves all the physiological and hemodynamic parameters in the early stages of lung injury in the model with significant improvement in the hypoxemia, early pulmonary hypertension and pulmonary microvascular injury seen in Ps controls.

2. Studies with Ranitidine

Disturbing reports with the utilization of long-term cimetidine in patients in intensive care units have been documented over the past few years (20,21). Mental status alterations have been observed with the drug and these are dose and time related. Impairment of hepatic and renal function greatly augment these changes. Mental deterioration has been shown to clear within 24 to 36 hours after stopping the drug. Because of these implications in ARDS patients who may already have impairment of renal and hepatic function, an alternative H_2 receptor blocker, ranitidine, which has no such side effects, was assessed in the experimental ARDS model. It has been postulated that in addition to its other actions, cimetidine functions as an oxygen-free radical scavenger and that this action might be contributary to the improvement seen with CID. Ranitidine has no such action. A series of studies were conducted using ranitidine in place of cimetidine in combination with diphenhydramine and ibuprofen.

3. Anti-platelet Activating Factor Studies

Platelet activating factor (PAF) is a lipid derived from the phospholipid component of cell membranes and is released from a variety of cells including

basophils, platelets, neutrophils, mast cells, endothelial cells and macrophages. It contributes to neutrophil margination and tissue edema formation. Administration of PAF in unanesthetized sheep resulted in pulmonary vasoconstriction and an increase in pulmonary lymph flow. A series of studies in the pseudomonas porcine model utilizing an experimental anti-PAF substance, SRI 63-675, were carried out.

4. Lipid Peroxidation Products

Toxic oxygen metabolites generated from activated neutrophils and monocytes have been implicated in the capillary endothelial injury seen in ARDS. These radicals are normally scavenged by endogenous superoxide dismutase and catalase; however, when the endogenous defense mechanisms are overcome by increased radical production, tissue damage occurs as a result of radical damage to the lipid component of cell membranes. Measurement of oxygen radical production in vivo is not possible at this time since superoxide has an extremely short half life. Radical production theoretically can be estimated indirectly by measurement of lipid peroxidation products such as malonaldehyde and conjugated dienes, i.e. compounds formed from the action of oxygen radicals on lipid membranes. Conjugated dienes have been found to be raised in the serum following endotoxin (22) or thermal injury (23). It was felt that measurement of these compounds in the porcine pseudomonas ARDS model would be a valuable indicator of oxidant injury and would reflect successful treatment with oxygen radical scavengers.

5. In Vivo Oxygen Radical Scavenger Studies

Numerous compounds have been shown in vitro to scavenge oxygen free radicals. We studied the effects of N-acetylcysteine (NAC), and a combination of superoxide dismutase and catalase (SODC) in the model. In the NAC group (NAC, N=7) the pigs were pretreated with 150 mgs/kg 1 hour i.v. bolus before

pseudomonas and followed by a maintenance dose of 20 mg/kg i.v. each hour. SOD-catalase group (SODC, n2) received SOD 2,000 units/kg continuous i.v. infusion over 3 hours and catalase 2000 units/kg i.v. bolus at 20 minute intervals over 3 hours. In addition to the intravenous administration of these compounds, studies were carried out in the model with the intraperitoneal administration of 50 mg/kg catalase given in three divided doses by i.p. injection 6, 12 and 18 hours (i.p. cat n=6) prior to infusion of Pseudomonas. Because catalase has a half life of 15-20 minutes in plasma, it was felt that adequate blood levels could be achieved by slow absorption following intraperitoneal administration rather than by continuous intravenous administration. This method of administration has been successfully carried out in sheep.

6. In Vitro Neutrophil and Macrophage Studies

Many investigators have established that human and various animal species produce toxic oxygen metabolites from neutrophils in vitro. Neutrophils became "activated" by contact with a soluble stimulus such as endotoxin or lipopolysaccharide and undergo a respiratory burst with subsequent production of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) in vitro. Studies documenting the kinetics of O_2^- and H_2O_2 release in addition to the total amount of these metabolites released from pseudomonas-primed porcine neutrophils from peripheral blood and from alveolar macrophages retrieved from bronchoalveolar lavage fluid are at present ongoing in this laboratory. Comparative studies between blood neutrophils and monocytes, and alveolar macrophages in the pre-pseudomonas phase and post-injury phase with regard to the rate and amount of O_2^- and H_2O_2 produced are being undertaken. Neutrophils and monocytes are separated from the pre- and post-injury blood samples and adjusted to a known concentration of cells per milliliter. For kinetic studies pre-injury and post-injury neutrophils are stimulated with phorbol myristate acetate (PMN) (a known white cell activator) in a dual beam spectrophotometer in the presence of

cytochrome C at 550 nm. The rate of superoxide dismutase inhibitable O_2^- production per minute is directly proportional to the rate of cytochrome C reduction. For quantitative studies, pre- and post-injury neutrophils and monocytes as well as pre- and post-injury bronchoalveolar lavage retrieved macrophages are plated onto a Biotech 12 channel microplate reader again using the reduction of cytochrome C as the indicator of superoxide production. H_2O_2 production is stimulated by horseradish peroxidase-dependent oxidation of phenol red by H_2O_2. Results are expressed as nmoles/well of the compounds produced.

7. Compliance Studies

Static and dynamic pulmonary compliance are important parameters in human ARDS as well as animal ARDS models. A method for estimating these parameters in the porcine model has recently become available to this laboratory. We are at present examining static and dynamic compliance in the injured animals compared to controls, and will then compare the effects of various treatment modalities on these parameters. Compliance measurements are taken in the following manner:

The pigs are intubated orotracheally with a cuffed endotracheal tube and mechanically ventilated to standardized tidal volume (Model 615; Harvard Apparatus Respirator, South Natick, MA) of 10 ml/kg and with 10 breaths/minute for dynamic lung compliance measurements, and periodically ventilated to a standard pressure (Mark 7 Respirator; Bird Corporation, Palm Springs, CA) of 30 cm H_2O for static lung compliance measurements. Esophageal pressure was measured with a balloon positioned in mid-esophagus where the changes in esophageal pressure were most negative. Airway pressure was measured from a side port of the ventilator tubing just proximal to the endotracheal tube connection. The airway and esophageal pressure catheters are connected across a differential

pressure transducer (Validyne MP45-4, range ± 50 cm H_2O; Validyne Engineering Corp., Northride, CA) for measurement of transpulmonary pressure. Airway pressure alone is also measured by connecting the airway pressure catheter to an identical pressure transducer referenced to atmospheric air (Model 8805B; Hewlett-Packard, Waltham, MA). Airflow is measured using a calibrated pneumotachygraph (Model 21071B; Hewlett-Packard, Waltham, MA) coupled to a flow transducer (Model 47304A; Hewlett-Packard, Waltham, MA). The airflow signal is sent to a respirator integrator (Model 8815A; Hewlett-Packard, Waltham, MA) for volume determination. Volume is electronically plotted against both airway and transpulmonary pressures (X-Y Recorder Module; Warren E. Collins Inc., Braintree, MA).

Both dynamic and static lung compliance measurements are taken respectively every 15 minutes starting just before the intravenous infusion of Pseudomonas in the treated group and just before the infusion of normal saline in the control animals (Time 0). Prior to each dynamic lung compliance measurement, PEEP is disconnected from the exhalation port of the Harvard ventilator and was replaced by the pneumotachygraph for volume determination. At least eight tidal volumes occur before each dynamic lung compliance measurement to ensure that the lungs have stabilized at the new FRC. Dynamic compliance is computed by dividing the peak inspiratory pressure by the tidal volume.

Following each dynamic lung compliance measurement, the Harvard ventilator is disconnected and replaced by the Bird respirator supplied with pressurized oxygen. The pneumotachygraph is placed at the distal end of the exhalation tubing. A hand-operated, spring-loaded, on-off valve (Model BE137; Instrumentation Industries Inc., Bethel Park, PA) introduced midway between the endotracheal tube connection and the pneumotachygraph prevents air from flowing except during designated points. Prior to each static lung compliance measurement, the lungs are twice fully inflated to TLC and allowed to rapidly deflate

(with the exhalation valve open) to ensure maximal alveolar recruitment. Measurements are recorded following the third inflation at which point small bursts of air are released from the lungs until FRC was reached. Intervals of at least 0.5 sec are inserted between each burst to ensure that pressures have stabilized. Typically, each static lung compliance measurement includes at least eight points along the curve. Static compliance is computed by taking the slope of the most linear portion of the curve above FRC.

RESULTS

1. RID

Six pigs were studied using the therapeutic combination of ibuprofen 125 mg/kg, diphenhydramine 10 mg/kg and ranitidine 25 mg/kg (RID) given i.v. at 20 and 120 minutes after the onset of pseudomonas infusion. RID therapy maintained SI, EVLW and PaO_2 at control levels while PAP progressively rose, becoming significantly above control at 90 minutes (Table 1). When compared with CID therapy, RID was found to be equally efficacious. In these acute animal studies, the effects of ranitidine and cimetidine as H_2 antagonists were similar. There was no evidence by the measured parameters of the reported ability of cimetidine to scavenge oxygen free radicals and thereby significantly improve the response to injury in this model.

2. Anti PAF SRI 63-675

Five pigs were studied using an experimental platelet activating (PAF) factor receptor blocker (Sandoz SRI 63-679) given intravenously in a dose of 40 mg/kg bolus at the start of Ps infusion. APAF prevented the early rise in PAP, the late fall in PaO_2 and the late increase in slope index. The deterioration in SAP and CI were delayed and SAP was significantly improved at 180 minutes compared to PS control animals. However, the increase in EVLW

improved in the APAF group (Table 2). This compound did, however, cause significant hemolysis which would preclude its use clinically in its present form.

3. Lipid Peroxidation Products

Plasma samples were taken at time 0 (control) and at five-minute intervals during and after termination of pseudomonas infusion in the model. The lipid fraction of the samples was extracted using the chloroform methanol method of Lunec and Dormandy. The extracted lipid component was dried in a water bath under nitrogen and the precipitated lipid was reconstituted in 1 cc methanol and measured in the dual beam spectrophotometer at 233 nm. This wavelength is not specific but is characteristic of conjugated dienes. In the septic animals (Ps, n=5) the conjugated diene levels became significantly increased above time 0 at 45 minutes ($p<0.05$) after the onset of pseudomonas and returned to control levels within five minutes (Table 3). Conjugated diene levels in the septic animals were significantly higher ($p<0.05$) than control animals given a saline infusion (C, n=6) at 35 and 40 minutes, but otherwise there were no differences between the groups (Table 3). It was felt that conjugated dienes would not be a reliable and reproducible indicator of oxygen radical damage to lipid membranes in this model. It is likely that these products are probably not produced in sufficient quantity in the porcine model to be adequately assayed when compared to small animals such as guinea pigs and rats which have a much higher metabolic rate and have been shown to produce large quantitites of conjugated dienes after injuries such as endotoxin or burns.

4. In Vivo Oxygen Radical Scavenger Studies

Pigs pretreated with N-acetylcysteine (NAC, n=3) showed no differences from control pseudomonas pigs (Ps, n=5) in any parameters. Pigs given intravenous infusion of a combination of superoxide dismutase and catalase (SODC, n=2)

were not significantly different from pseudomonas controls. In the group treated with intraperitoneal catalase 50 mg/kg (i.p. CAT, n=6) only SAP was significantly improved over control pseudomonas animals (Ps, n=6) at 90, 120, 180, 240 minutes (Table 4). EVLW was not improved at any time over Ps animals. SI was significantly improved in the i.p. catalase group compared to Ps controls at 105 minutes post-Ps infusion, however, since neither lung water PaO_2, CI were not significantly better it is likely that this perceived improvement in SI was artifactual. The explanation for this is not clear but it may be that a large molecule like catalase interferes with the scintigraphic technique in some way.

5. In Vitro Neutrophil and Macrophage Studies

Data collected from our studies thus far indicate that neutrophils recovered from blood in the post-pseudomonas phase in the model are "primed" for the release of superoxide. This was determined by comparing the activation lag times of pre-pseudomonas and post-pseudomonas neutrophils. Pre-injury neutrophils began to produce superoxide approximately 2 minutes after activation with PMA in vitro. Post-injury cells require less than a minute to produce O_2^- after PMA (Fig. 1). These post-injury cells have a high initial rate of production compared to pre-injury cells. Endpoint production studies of O_2^- and H_2O_2 are at present ongoing but it would appear that the pre-injury cells overall generate more superoxide and H_2O_2 than the post-injury cells (Fig. 2). It seems likely that the reason for this is that the "primed" post-injury cells have already produced oxygen free radicals in considerable amounts and are depleted of superoxide and H_2O_2 when harvested after the bacteremia has been in progress for some time. Initial studies on bronchoalveolar macrophages harvested before and two hours after the onset of pseudomonas indicate that in contrast to post-injury arterial PMN's, the post-injury macrophages produce more superoxide

and H_2O_2 than pre-injury cells (Fig. 3). This difference would appear to be more marked if the macrophages are harvested at five hours post-injury (Fig. 4). These findings although preliminary at this stage, imply that the neutrophils are the main culprit in the early stage of the lung injury seen after Ps infusion and also that the macrophages take longer to become activated and may well play a significant role in the later stages of the injury. Certainly, it would appear that these cells have the ability to produce large quantities of superoxide and H_2O_2 when primed in vivo. As yet, no studies have been performed in control animals and statistical comparisons will not be possible until all the studies are completed.

6. Compliance Studies

Both static and dynamic compliance decreased significantly within the first hour after the onset of pseudomonas infusion (Fig. 5). This precipitous drop was maximal in the first 15 minutes post-Ps and continued to decline until the Ps infusion was stopped at one hour. A consistant short-lived significant improvement was seen immediately on stopping the bacteria but thereafter compliance decreased and remained decreased throughout the remainder of the study until 300 minutes. These compliance changes in the group studied thus far (Ps com., n=5) correlate well with changes in oxygenation and lung water in the model. Static and dynamic compliance show similar and parallel changes over time (Fig. 5). Saline control animals are at present being studied to monitor the effects of anesthesia and positioning on compliance in the model. From preliminary results, it would seem that these variables have little or no effect on compliance. Compliance measurements will be an important tool in future studies on the effects of therapeutic intervention in the model.

Studies with Pulmonary Endothelial Cell Monolayers

In addition to these in vivo and in vitro studies, we have begun to establish an in vitro porcine pulmonary artery endothelial cell line. By

utilization of this cell line and radioactive labeling of neutrophils, monocytes and macrophages, it should be possible to establish the degree of endothelial cell damage and permeability and also the adherence phenomena of stimulated and unstimulated circulating cells. Projected studies will include compounds which in theory prevent neutrophil adherence and subsequent endothelial damage, the effects of ibuprofen, cimetidine and various other anti-inflammatory compounds on white cells and endothelial cells. These studies should clarify previous in vivo work and lay the foundation for further in vivo studies. We also hope to grow pulmonary microvascular endothelial cells for similar studies.

CONCLUSIONS

The lung injury in ARDS is mediated by many different substances, many of which may not yet be known. Histamine, prostaglandins, oxygen free radicals, small molecular weight biologially active lipids, interleukins, tumor necrosis factor, proteolytic enzymes have been implicated in the injury in in vitro and in vivo experiments. We have already established in the porcine pseudomonas model that treatment with a combination of H_1 and H_2 blockers and a cyclooxygenase inhibitor can attenuate all phases of the injury, particularly the early phase of pulmonary hypertension as well as the late permeability phase.

We have established that treatment with an anti-platelet activating factor SRI 63-675 is effective in preventing the early phase of the injury but does not seem to be as effective in preventing the permeability phase. The effects of this compound are very similar to ibuprofen. When platelet-activating factor was infused into sheep, the levels of TxB_2 rose significantly and was associated with a rise in PAP. Cyclooxygenase inhibitors prevented this rise in TxB_2 and in PAP. It appears that thromboxane plays an important role in PAF mediated, endotoxin and pseudomonas lung injury. At the present time, the

anti-platelet activating factor compound SRI 63-675 causes severe hemolysis and would not have clinical applications.

In vivo studies with oxygen radical scavengers in this laboratory have been inconclusive and generally disappointing. It is becoming clear from in vitro work that oxygen radicals do have a role in the injury but how this can be prevented is not yet apparent. The administration of exogenous radical scavengers in this model system have not altered the injury in any significant way although some success has been reported in rats, guinea pigs and sheep. Part of the difficulty in assessing these compounds is that there is no good marker of oxidant injury in serum or in tissue. In the porcine model, serum levels of conjugated dienes as a measure of lipid peroxidation by O_2 radicals have been disappointing. In thermally injured guinea pigs, however, raised levels of conjugated dienes measured in serum correlate well with other parameters of injury and the production of these products is inhibited by a combination of radical scavengers (24). The porcine model does not produce conjugated dienes in sufficient quantity to be a reliable indicator of oxidant activity.

We have established in this model that pseudomonas "primed" neutrophils produce superoxide radicals at a much greater rate than in control animals, thus implicating neutrophils as a major injury cell in the early phase of the process. However, it appears from some preliminary results that alveolar macrophages may also be implicated in the injury but that priming of these cells takes longer than neutrophils. It may be that macrophages, in addition to producing interleukins and tumor necrosis factor, also produce superoxide and hydrogen peroxide and are involved in the later stages of an injury.

Studies are ongoing with compounds which prevent neutrophil margination and adherence to the endothelium and thus prevent release of inflammatory mediatiors.

It is hoped that these compounds will limit the extent and the severity of injury. Assays of tumor necrosis factor and the interleukins are also ongoing. Tumor necrosis factor increases significantly shortly after the onset of pseudomonas infusion in the model from data analyzed thus far and appears to play a role in the early phase of the injury.

Both static and dynamic compliance decrease dramatically after the onset of pseudomonas infusion and remain decreased throughout the study period. Saline control studies examining the effects of anesthesia and positioning on the animals are at present ongoing. Compliance will be an important parameter in the future evaluation of therapeutic intervention in porcine pseudomonas ARDS.

RECOMMENDATIONS

ARDS in both civilian and military patients carries a considerable mortality. The syndrome often occurs in previously fit individuals who undergo direct pulmonary as well as non-pulmonary injuries. It has been established that the lung injury seen in ARDS is due to many inflammatory mediators. Elucidation of these mediators and pathways are therefore likely to be necessary for adequate pharmacological intervention and treatment in the syndrome. We have established in animal models that a combination of H_1 and H_2 blockers and a cyclooxygenase inhibitor significantly improve all parameters but do not completely abolish the syndrome. We know that an anti-platelet activating factor compound can partially ameliorate certain phases of the injury. Continued efforts at elucidation and identification of the various inflammatory mediators, particularly substances secreted by neutrophils and macrophages and pharmacological blockade of their actions will be the purpose of further research in this laboratory.

Table 1: RID vs. CID

	PaO_2 (torr)		EVLW ml/kg		SI x 10^{-30}/mm	
Time	15	180	0	180	75	165
Control	185 ± 6.2	205 ± 15**	4.1 ± 0.9	4.3 ± 1.2**	0.43 ± 0.05	0.38 ± 0.09**
Ps	167 ± 20	93 ± 17	6.8 ± 0.6	14.4 ± 2.2	1.80 ± 0.16**	2.05 ± 0.23
CID	212 ± 8.2	208 ± 40**	6.3 ± 0.4	7.6 ± 0.3**	0.96 ± 0.24**	0.37 ± 0.42**
RID	215 ± 2.3	218 ± 22**	4.7 ± 0.9	7.2 ± 1.1**	1.37 ± 0.18*	0.66 ± 0.34**

*p<0.05 USC; **p<0.05 vs. Ps; mean ± SEM

Table 2: Effects of SRI 63-675 (An Anti-PAF Agent)

		Control	Ps	APAF
PaO_2	0	174 ± 9	233 ± 16	210 ± 16
	180	203 ± 14*	104 ± 19	187 ± 19*
EVLW	0	4.1 ± 1	6.5 ± 1	6.4 ± 1
	180	4.3 ± 1	11.3 ± 2	10.3 ± 0.8
PAP	0	14 ± 2	17 ± 2	17 ± 2
	30	16 ± 3*	46 ± 3	20 ± 2*
	180	12 ± 2*	37 ± 3	30 ± 4
CI	0	2.2 ± 0.1	3.7 ± 0.4	2.8 ± 0.2
	180	2.8 ± 0.2*	2.1 ± 0.2	2.0 ± 0.2
SAP	0	107 ± 8	124 ± 3	110 ± 5
	180	128 ± 7*	78 ± 7	89 ± 18*
SI	15	0.43 ± 0.05	0.48 ± 0.4	1.02 ± 0.4
	105	0.37 ± 0.08*	1.71 ± 0.6	0.3 ± 0.39*

Mean ± SEM; $p \leq 0.05$ vs. Ps

Table 3: Plasma Level of Conjugated Dienes at 233 nm

Time (mins)	0	30	35	45	60	90
Control	0.9 ± 0.2	0.8 ± 0.05	0.8 ± 0.08	0.9 ± 0.2*	0.9 ± 0.1	0.8 ± 0.1
Pseudomonas	0.9 ± 0.1	1.0 ± 0.1**	1.0 ± 0.1**	1.1 ± 0.3	0.9 ± 0.2	0.9 ± 0.1

Mean ± SD; *From within group control; **From control group ($P<0.05$)

Table 4: Effects of Intraperitoneal Catalase

	SAP	PAP	CI	PaO_2	EVLW
IPCAT					
0	127 ± 5	21 ± 1	3.1 ± 0.3#	241 ± 19#	7.4 ± 0.6
30	140 ± 11	57 ± 3*#	2.3 ± 0.2	252 ± 0	---
60	151 ± 6	47 ± 2*#	2.9 ± 0.3	168 ± 21	7.2 ± 1.0#
120	140 ± 7°	35 ± 3*#	2.7 ± 0.2	174 ± 28	8.6 ± 1.7#
180	144 ± 7°	38 ± 3*#	2.5 ± 0.2	166 ± 26	10.0 ± 0.9#
Saline Control					
0	107 ± 8	15 ± 2	2.3 ± 0.2	174 ± 9	4.1 ± 0.1
30	126 ± 7	17 ± 3	2.7 ± 0.2	201 ± 22	3.3 ± 1.7
60	128 ± 7	17 ± 2	2.6 ± 0.2	196 ± 10	3.7 ± 1.2
120	139 ± 4	15 ± 3	2.8 ± 0.4	186 ± 12	4.3 ± 0.8
180	128 ± 3°	13 ± 2	2.8 ± 0.2	203 ± 15	4.3 ± 1.2
Pseudomonas Control					
0	124 ± 1	17 ± 2	3.7 ± 0.4	232 ± 16#	6.4 ± 1.0
30	117 ± 9	46 ± 3*#	2.5 ± 0.5*	190 ± 26	7.0 ± 0.3#
60	105 ± 5	43 ± 3*#	2.9 ± 0.3*	150 ± 24*	8.7 ± 0.6#
120	84 ± 6*#	35 ± 2*#	2.5 ± 0.1*	146 ± 18*	8.9 ± 0.2#
180	78 ± 3*#	37 ± 3*#	2.1 ± 0.2*	104 ± 19*#	11.3 ± 2.0*#

* vs. time 0; #vs. control; ° vs. chips

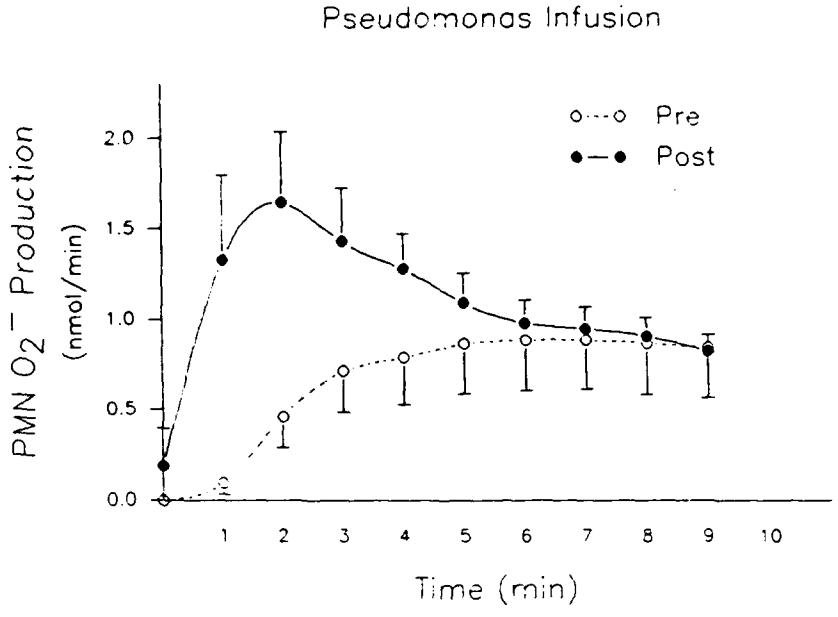

Figure 1: Rate of superoxide production from neutrophils isolated from plasma in the pre- and post-pseudomonas infused pigs.

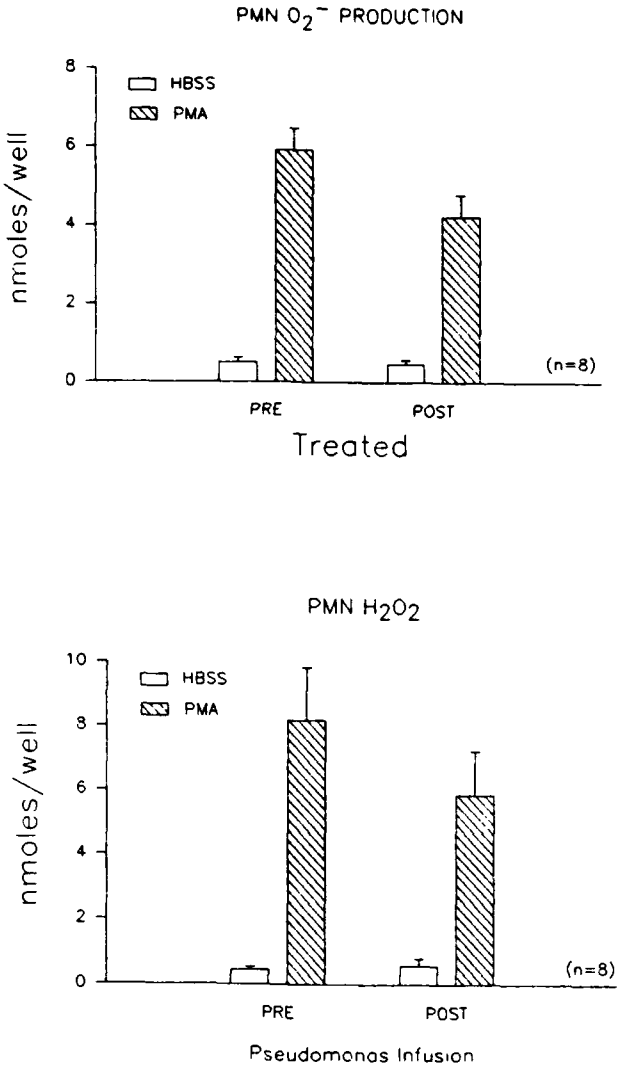

Figure 2: Superoxide anion and hydrogen peroxide production from neutrophils isolated from plasma in the pre- and post-pseudomonas phases in the model.

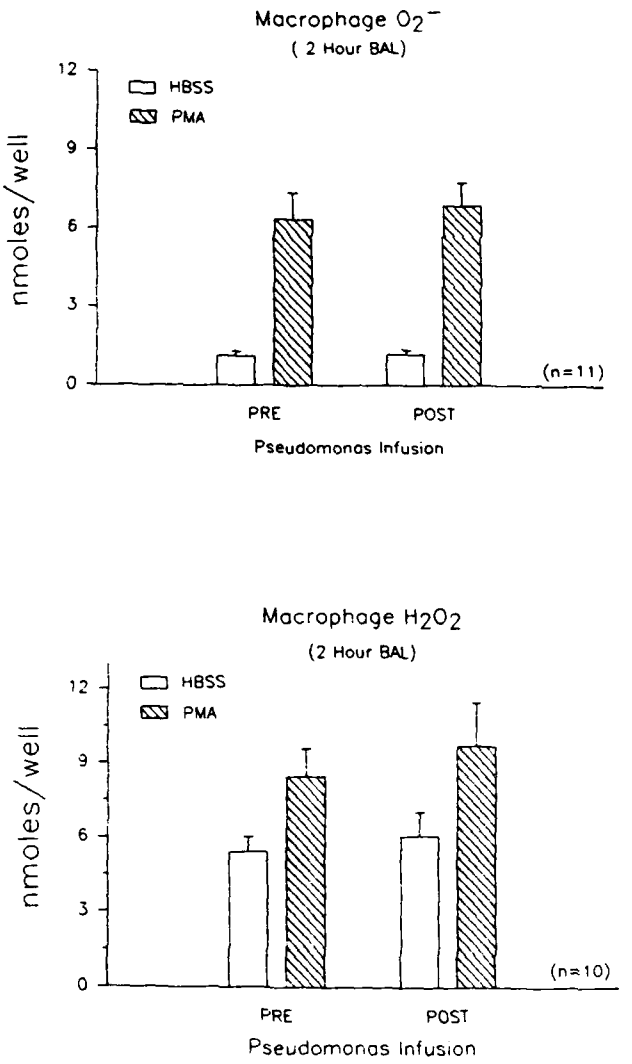

Figure 3: Superoxide and H_2O_2 production from bronchoalveolar lavage retrieved macrophages in the pre- and two hours post-pseudomonas stages of the injury in the model.

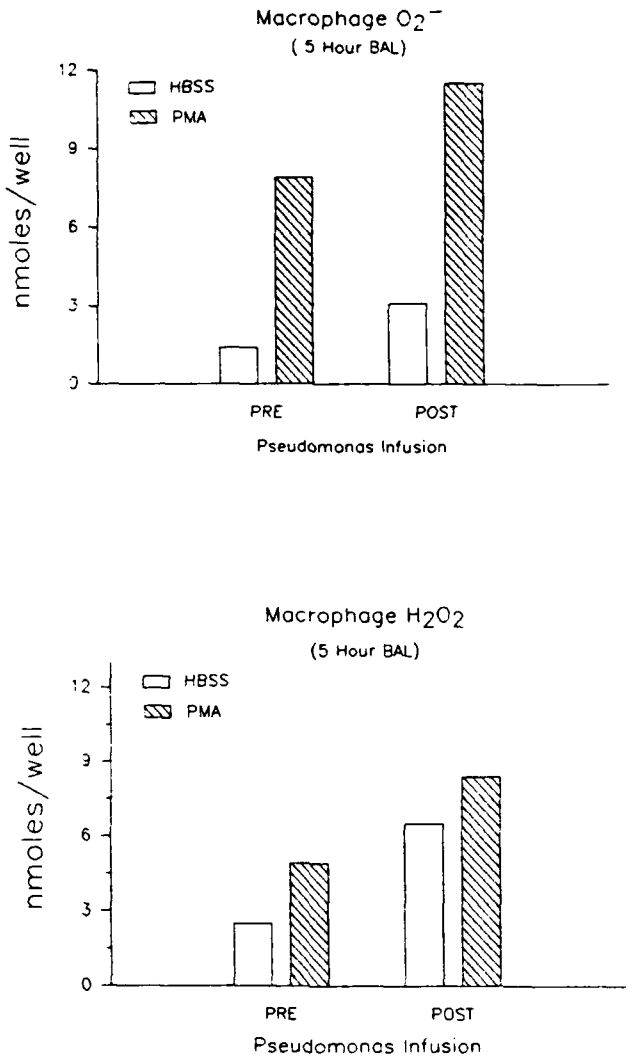

Figure 4: Superoxide and hydrogen peroxide produced from bronchoalveolar lavage retrieved macrophages in the pre- and five hours post-pseudomonas phases of the injury.

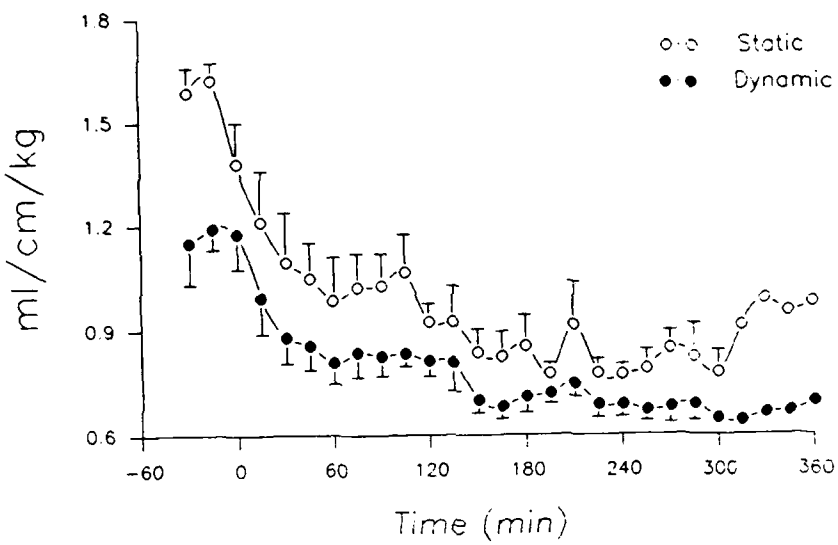

Figure 5: Static and dynamic trans pulmonary compliance changes before and after pseudomonas infusion (n=6).

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